ENDOTHELINS (ET) are a family of 21-residue polypeptide hormones first isolated, sequenced, and cloned by Yanagisawa et al. (25). ET-1 is a well recognized and potent constrictor in many mammalian vessels (9, 10). When injected into mammals in vivo, ET-1 often evokes transient hypotension followed by more long-lasting hypertension (20). This biphasic response was generally attributed to the initial release of endothelium-derived relaxing factor, most probably nitric oxide, from endothelial cells (6, 24) followed by a direct ET contraction of vascular smooth muscle cells (19). Mammalian ET-1 has also been found to have significant cardiovascular effects in fish (5, 15, 16, 21). Olson et al. (15) observed that a bolus injection of 667 pmol/kg body wt ET-1 into the dorsal aorta of Oncorhynchus mykiss produced a triphasic pressor-depressor-pressor response. Although this approach demonstrated the trout cardiovascular reactivity to ET-1, it did not clarify the mechanism behind the changes in dorsal aortic pressure (PDA).

Branchial and systemic vessels are in series in teleosts. Therefore, changes in PDA can be caused by many markedly different cardiovascular perturbations, such as systemic constriction, branchial dilation, or increased cardiac output (CO) as a result of increased venous return. As an example, angiotensin II and urotensin II increase trout PDA by increasing systemic vascular resistance (R_s; 8, 13), arginine vasotocin increases PDA by increasing both systemic vascular resistance and venous return (2), and epinephrine increases PDA by increasing systemic vascular resistance and venous return and decreasing gill resistance (R_g; 27). Therefore, further evaluations using fish outfitted with cannulas to monitor PDA, ventral aortic pressure (PVA), and central venous pressure (PVe) while concurrently measuring CO are necessary to determine the site(s) of the systemic pressor activity of ET in trout.

In the present study, PVA, PDA, PVe, and CO were measured in unanesthetized trout during the injection or infusion of mammalian ET-1. Because results from these experiments indicated that ET-1 may also affect venous pressure, an additional series of experiments were conducted to specifically evaluate the effect of ET-1 on venous function. The recent availability of trout ET (tET; Ref. 23) permitted comparison of the effects of mammalian and homologous tET in trout. Both ET-1 and tET were found to be potent branchial constrictors, and both peptides showed dose-dependent modulation of cardiovascular function. ET-1 was also found to affect venous function by decreasing vascular compliance (VC), whereas it did not affect unstressed blood volume (USBV). These findings lend strong support for involvement of ET in hemodynamic regulation and especially implicate branchial resistance vessels as important effectors of ET's actions in trout.
and systemic capacitance vessels as effector sites for the hormone.

**MATERIALS AND METHODS**

Animals. Rainbow trout (Oncorhynchus mykiss, mixed Kamloops strain; 0.3–0.8 kg) of either sex were purchased from a local hatchery and kept in circulating 2,000-liter tanks at 12°C and under appropriate seasonal light-dark cycles. Fish were fed a maintenance diet of commercial trout pellets (Purina) up to 48 h before experiment.

Pressure flow experiment. Methods for cannulation of the ventral and dorsal aortas and ductus Cuvier and placement of a transonic flow probe have been described in detail (14). Trout were anesthetized in benzocaine (ethyl-p-aminobenzoate; 1:12,000, wt/vol) before surgery. The dorsal aorta was cannulated percutaneously through the roof of the buccal cavity with heat-tapered polyethylene tubing (PE-60); the gills were not irradiated during this brief (<1 min) procedure. Thereafter, gills were continuously irrigated with 10°C aerated water containing 1:24,000 NaCl benzocaine during placement of other cannulas and the flow probe. The pericardial cavity was exposed with a midline ventral incision, and the right horn of the ductus Cuvier and the ventral aorta were nonocclusively cannulated with 5-cm-long, 0.51-mm-ID silicone tubing (Dow Corning veterinary grade; Königsberg Instruments, Pasadena, CA). The free ends of the cannulas were connected to 60 cm of PE-90 tubing. All cannulas were filled with heparinized saline (100 USP units/ml heparin in 9.0 g/l NaCl) and connected to Gould P23 pressure transducers. A 3S Transonic flow probe (Transonic Systems, Ithaca, NY) was placed around the ventral aorta, distal to the site of the cannula insertion, and connected to a Transonic T206 flowmeter. The ventral incision was closed with interrupted silk sutures and sealed with cyanoacrylate gel (Super Glue gel). The venous (ductus Cuvier) and ventral aorta cannula and flow probe lead were exteriorized and secured to the fish with silk sutures. The fish were revived and placed in black plastic tubes immersed in a 1,500-liter experimentation aquarium with aerated, through-flowing well water at 12°C. Experiments were conducted 24 h after surgery, and a four-way stopcock in the dorsal aortic or venous cannulas served as the site for drug injection and/or infusion.

Analog pressures were recorded with Hewlett-Packard 7853A patient monitors (Palo Alto, CA). Digitized signals of pressure and flow were collected at 0.1-s intervals, and 1-s averages were stored on computer. The pressure transducers were calibrated with a water manometer, and the flowmeter was calibrated in situ at the end of the experiment by pump perfusion of the ventricle with 12°C saline at known flow rates. Heart rate (HR) was derived from the CO pulse flow. R$_S$ and R$_G$ were calculated by dividing the respective pressure gradients by CO, i.e., $R_S = (P_{DA} - P_{VEn})/CO$ and $R_G = (P_{VA} - P_{DA})/CO$. Stroke volume (SV) was calculated from CO and HR: SV = CO/HR.

Resting pressures and CO were monitored for 1–2 h before experimentation to ensure stability; control parameters were recorded for a minimum of 5 min before infusion. ET-1 was infused for 30 s at a rate of 0.3 ml/min to clear the dorsal aortic cannula (~0.25 ml volume) and to provide a small priming dose and then at a rate of 1 ml/kg body wt 1·min$^{-1}$ for 1 h. The infusion doses were 0.1, 1, and 3 pmol·kg$^{-1}$·min$^{-1}$ into the dorsal aorta and 0.3 and 1 pmol·kg$^{-1}$·min$^{-1}$ into the ductus Cuvier of ET-1. Bolus doses of 100, 333, and 667 pmol/kg body wt ET-1 and 100, 333, and 1,000 pmol/kg body wt of tET were administered into the dorsal aorta followed by a 0.3 ml saline flush. Data were recorded for 1–2 h after the bolus was injected or after infusion was terminated.

Vascular capacitance. Vascular capacitance curves were constructed from measurements on conscious trout in vivo using the ventral aorta occlusion method to establish zero-flow CO conditions as described previously (27) and summarized below. Trout were anesthetized in benzocaine, and the dorsal aorta and ductus Cuvier were cannulated as described above; the cannulas were filled with heparinized saline. An inflatable vascular occluder was fitted around the distal bulbous and ventral aorta, and the venous cannula and occluder tubing were exteriorized and secured to the fish. Details on the construction of the occluder can be found in Zhang et al. (27). The fish were revived and placed in black plastic tubes and immersed in holding tanks. Experiments were conducted 24 h after surgery. P$_{DA}$ and P$_{VEn}$ were measured in unanesthetized fish before and during occlusion of the ventral aorta. Inflation of the occluder (~5 s duration) produced a rapid decrease in P$_{DA}$ and increase in P$_{VEn}$, indicating zero-flow CO; P$_{VEn}$ was assumed to be mean circulatory filling pressure (MCFP) at this point. Ventral aortic occlusion was then released, and the blood pressure reached preocclusion levels within two or three consecutive pressure-volume data points. Vascular capacitance curves were obtained by measuring MCFP at 80, 90, 100, 110, and 120% of normovolemic blood volume (assumed to be 35 ml/kg body wt). Whole blood from a donor fish was used for volume expansion and all volume adjustments were made via the dorsal aorta cannula. Ventral aortic occlusion was initiated within 30 s after each blood volume perturbation, and the volume was restored to normovolemia within 30 s after pressure measurement. The order of volume withdrawal or addition was randomized. The interval between each volume perturbation, during which the fish were normovolemic, was 15 min. Thus the fish were exposed to hyper- or hypovolemia for only a short portion of the experiment. During the control period, the fish were infused with saline at 0.25 ml/h. The fish were then infused at the same flow rate with ET-1 (1 pmol·kg$^{-1}$·min$^{-1}$), and the entire pressure-volume protocol was repeated. ET-1 was infused for a minimum of 30 min before capacitance measurements. All saline and ET-1 infusions were made via the venous cannula.

Two vascular capacitance curves were determined from the blood volume-MCFP relationships for each fish: one during saline infusion and a second during infusion of ET-1. Capacitance curves are not linear (26), therefore VC and USBV were determined at three blood volume intervals: 80–100%, 90–110%, and 100–120% by regression analysis of the three consecutive pressure-volume data points within each interval. By convention, MCFP was treated as the independent variable; therefore, the slope of the resultant volume-pressure line was equal to VC (Δvolume/Δpressure), and the intercept of this line with the blood volume axis at MCFP = 0 was assumed to be the percentage of the total blood volume in the unstressed compartment (18). This percentage, multiplied by the actual blood volume, equals the predicted USBV. The product of percent blood volume times estimated actual blood volume (35 ml/kg body wt) permitted conversion of VC and USBV into actual volumes, i.e., milliliters per millimeter of mercury per kilogram and milliliters per kilogram, respectively.

Statistics. Comparisons were made with appropriate paired or unpaired Student's t-test or repeated-measures ANOVA. Significance was assumed at $P < 0.05$. Values were expressed as means ± SE or means ± SE in Figs. 1–5, 8, and 9.

**RESULTS**

Cardiovascular effects. The effects of a bolus injection of 667 pmol/kg ET-1 on cardiovascular parameters in
unanesthetized trout are shown in Fig. 1. ET-1 injection caused a triphasic (pressor-depressor-pressor) response in PDA. The ET-1 bolus transiently increased PVA, PVen, pressure (mmHg) in ventral aorta, dorsal aorta, and ductus Cuvier, respectively; CO, cardiac output (ml·min⁻¹·kg body wt⁻¹); HR, heart rate (beats/min); SV, stroke volume (ml/beat); RG and RS, gill and systemic resistance (mmHg·min⁻¹·kg body wt⁻¹·ml⁻¹), respectively.

Infusion of ET-1 into the dorsal aorta (1 and 3 pmol·kg⁻¹·min⁻¹) increased PVA and RG, whereas the other parameters were unaffected. Infusion of 3 pmol·kg⁻¹·min⁻¹ markedl increased PVA, PDA, PVen, RG, and RS and decreased CO and SV. HR did not change.

ET-1 infusion into the ductus Cuvier at a rate of 0.3 and 1 pmol·kg⁻¹·min⁻¹ of ET-1 (Figs. 4 and 5) had a greater effect on the cardiovascular parameters of unanesthetized trout than did comparable doses infused into the dorsal aorta. Infusion of 0.3 pmol·kg⁻¹,
min\(^{-1}\) (Fig. 4) increased P\(_{\text{VA}}\), R\(_{G}\), and P\(_{\text{VEN}}\) although the P\(_{\text{VEN}}\) response was an apparent artifact of the infusion. At 1 pmol·kg\(^{-1}\)·min\(^{-1}\), every parameter except P\(_{\text{DA}}\) was affected; P\(_{\text{VA}}\), P\(_{\text{VEN}}\), R\(_{G}\), and R\(_{S}\) increased, whereas CO and SV decreased.

Vascular capacitance. The relationships between blood volume and P\(_{\text{DA}}\) during saline or ET-1 infusion and under normal or zero-flow CO are shown in Fig. 6. Blood volume expansion in saline-infused controls significantly increased P\(_{\text{DA}}\) at 110 and 120% of normal blood volume by ~15 and 30%, respectively. The effects of ET-1 infusion on both resting and zero-flow P\(_{\text{DA}}\) were greatest when blood volume was expanded to 120%; here, P\(_{\text{VEN}}\) was nearly doubled (not shown). Blood volume depletion below 100% did not affect arterial pressure, whereas P\(_{\text{VEN}}\) continued to fall in an almost linear relationship. Ventral aorta occlusion lowered P\(_{\text{DA}}\) from 23.7 ± 0.3 to 16.5 ± 0.3 mmHg (n = 7; P ≤ 0.05; Fig. 6) and raised P\(_{\text{VEN}}\) from 3.0 ± 0.2 to 4.1 ± 0.3 mmHg (P ≤ 0.05) at 100% blood volume. During zero-flow CO, the effects of blood volume perturbation on P\(_{\text{DA}}\) and P\(_{\text{VEN}}\) in saline- and ET-1-infused fish were similar to
the changes observed before the ventral aorta occlusion, albeit at lower pressure. In addition, it was interesting to note that the pre- and postocclusion $P_{DA}$ differences were less at greater blood volumes.

Capacitance curves for ET-1 infusion and control saline infusion are shown in Fig. 7, and USBV and VC calculated from capacitance curves of individual fish are listed in Table 1. ET-1 infusion produced a clockwise rotation of the compliance line (Fig. 7). This rotation reflects a decreased VC by ET-1 at all blood volumes. USBV calculated from the regression of the capacitance curves at 80–100%, 90–110%, and 100–120% of normal blood volume was unaffected by ET-1 (Table 1).

Because of limited availability, tET was administered only as bolus injection into the dorsal aorta of unanesthetized trout using 333 and 1,000 pmol/kg doses (Figs. 8 and 9, respectively) to bracket the initial 667 pmol/kg bolus of ET-1. Both 333 and 1,000 pmol/kg increased $P_{VA}$, $P_{DA}$, $P_{VEn}$, $R_G$, and $R_S$ and decreased CO. The 1,000 pmol/kg dose also decreased HR and SV. Furthermore, the 1,000 pmol/kg bolus produced a tri-
Table 1. Effects of ET-1 infusion on unstressed blood volume and vascular compliance in unanesthetized trout

<table>
<thead>
<tr>
<th>Unstressed Blood Volume, ml/kg body weight</th>
<th>Vascular Compliance, ml·mmHg⁻¹·kg body wt⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>90–110%</td>
<td>100–120%</td>
</tr>
<tr>
<td>80–100%</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td></td>
</tr>
<tr>
<td>26.0 ± 0.5</td>
<td>20.3 ± 0.7</td>
</tr>
<tr>
<td>ET-1</td>
<td>23.8 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>19.1 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>25.2 ± 1.1</td>
</tr>
</tbody>
</table>

|                                            | 90–110%                                  |
|                                            | 80–100%                                  |
|                                            | 100–120%                                 |
|                                            | 2.6 ± 0.1                                |
|                                            | 2.4 ± 0.1*                               |
|                                            | 3.56 ± 0.2*                              |
|                                            | 2.15 ± 0.2*                              |

Values are means ± SE (n = 7). ET-1, endothelin-1 (1 pmol·kg⁻¹·min⁻¹). *Significantly different (P ≤ 0.05) from saline infused.

DISCUSSION

The present study shows that ET-1 is a potent pressor peptide in trout and that it has dose-dependent effects on the trout cardiovascular system at a number of foci, including branchial and systemic resistance vessels and systemic capacitance veins. Qualitatively similar responses are produced by the recently sequenced tET, and these provide the first assessment of the cardiovascular effects of homologous ET in any fish and they affirm the validity of the present and prior studies performed on trout when only the heterologous peptide was available.

Previous studies showed that a 667-pmol/kg bolus of ET-1 produces a triphasic pressor-depressor-pressor response in the trout dorsal aorta (15), and this was confirmed in the present study (Fig. 1). The triphasic response did not occur in either the ventral aorta or central veins, therefore, it was the result of disproportionate changes in branchial and systemic resistance. Figure 1 shows that there are at least two factors that contribute to the PDA response: 1) ET-1 is a more potent branchial than systemic constrictor and 2) convection of the ET-1 bolus results in temporal stimulation of distinct resistance elements. Considering the ET-1 bolus increased R₉ by 10-fold, whereas R₅ only doubled supports the first contention. Enhanced branchial responses were also observed after ET-1 infusion (Figs. 2–5) and a tET bolus (Figs. 8 and 9). Furthermore, it was not uncommon for low doses of ET-1 to affect R₉ but not R₅ (Figs. 2 and 4). Temporal distribution of the ET-1 bolus could have contributed to the triphasic PDA in the following way: ET-1 injection into the dorsal aorta would result in initial exposure of the systemic resistance vessels and thereby produce an initial rise in PDA. After the ET-1 traverses the systemic circulation, it is delivered to the gills where it has an even greater effect on R₉. This results in an increase in R₉-to-R₅ ratio, thereby increasing Pᵥₐ and lowering PDA. The source of the third pressor phase is less obvious and may result from a combined increase in CO and a reduced R₉-to-R₅ ratio. Alternatively, the third phase may be due to a baroreceptor response to the preceding systemic hypotension, as is known to occur after bradykinin injection (14). Infusion of ET-1 obviates these temporal transients and prevents the triphasic response.

Infusion of ET-1 into the ductus Cuvier had a greater effect on Pᵥₐ than did ET-1 infusion into the dorsal aorta (Figs. 2 and 5), presumably because there was less opportunity for dilution or tissue inactivation of the peptide.
ET-1 before it entered the gills (12). However, high ET-1 doses increased $P_{VA}$ irrespective of injection mode (Figs. 1, 3, 5, 9). In most instances, as $P_{VA}$ increased CO fell. The decreased CO was most consistently correlated with a decreased SV, the latter resulting from an excessive afterload (11). Although bradycardia was observed during an ET-1 bolus, it did not occur during ET-1 infusion and, therefore, HR appears to be a secondary reflexive response to the hypertension rather than a contributory determinant of CO. Similarly, the increased $P_{Ven}$ would be expected to increase, not decrease, CO. Thus the effects of ET-1 on $P_{Ven}$ can be explained by a combined decrease in venous compliance (see below) and decreased ventricular ejection. The excessively elevated afterload produced by ET-1-mediated increases in $R_G$ undoubtedly contributed to the elevated $P_{Ven}$, however, ET could also have direct effects on the venous circuit. In fact, trout veins in vitro are nearly 10 times more sensitive than trout arteries to tET (23). Although it is technically difficult to examine venous function in vivo, this can be accomplished with vascular capacitance curves (7, 17, 18) and the method has been successfully applied to trout (14, 26, 27). Figure 7 and Table 1 show that ET-1 decreases VC at all blood volumes, whereas it does not substantially effect venous tone. Decreased venous compliance will increase $P_{Ven}$, thereby increasing venous return and CO. The physiological significance of an increased venous return is unclear in view of the seemingly discordant increase in $R_G$; however, lower and perhaps physiological levels of plasma ET may permit selective regulation of the more sensitive venous system.

Central venous pressure, and therefore venous return and CO in mammals, can be increased by either an increase in venous tone or a decrease in venous compliance, and these two factors are not necessarily mutually inclusive (7, 17, 18). It has also been observed in trout that vasoactive hormones may act independently on tone and compliance, i.e., arginine vasotocin increases tone without affecting compliance (2), atrial natriuretic peptides increase compliance but do not generally affect tone (14), whereas epinephrine simultaneously increases tone and decreases compliance (27). In mammals, the actions of ET-1 on MCFP are indirectly mediated by the sympathetic nervous system (22); this does not appear to be the case in trout, however, as ET-1 only affects venous compliance (Table 1; Ref. 27).

The relative physiological impact of venous compliance and venous tone changes as blood volume changes. The effects of venous compliance on central venous pressure are minor at low blood volumes but become more important as blood volume is expanded, whereas venous tone uniformly affects venous pressure at all blood volumes (3). This implies that the physiological action of endogenous ETs on the venous system of trout is designed to either enhance central venous pressure as blood volume is expanded or to minimize the contribution of ET to venous pressure during hypovolemic states.

Although the first inclination is to assume that the effects of ET-1 on venous pressure are orthograde, i.e., they are important determinants of venous return and CO (perhaps to counter increased $R_G$ and $R_S$), it may also be important to consider the retrograde effects of elevated venous pressure, especially as they pertain to the kidney. Brown and Amer (1) showed that ET-1 has potent antidiuretic actions in the in situ perfused trout kidney, and it could be inferred from their studies that ETs promote volume expansion in freshwater trout. However, in intact freshwater trout, ET-1 infusion did not produce antidiuresis (15). This discrepancy can be
explained by an inability to control venous pressure in the perfused preparation and/or the volume status of the intact fish. Thus, in the perfused preparation, venous pressure is equal to atmospheric pressure and not controlled (1) and the effect of ET-1 on renal preglomerular arterial vasoconstriction, hence glomerular filtration rate (GFR), will be unopposed by postglomerular blood pressure. However, it is possible in intact fish that the concomitant increase in renal venous pressure offsets preglomerular constriction and GFR is sustained. In this context, the importance of ET-1 on venous compliance becomes evident. During hypovolemia, ET-1 has minimal effect on venous pressure, and the antidiuretic decrease in GFR is unopposed by venous pressure and volume is restored. However, as trout become volume expanded, the effects of ET-1 on venous pressure increase and GFR may be sustained, or may even be increased, during hypervolemia.

In our companion study (23), we isolated tET from trout kidney because the ET-1 immunoreactivity was highest in this tissue. It is not known whether renal ET is due to tissue accumulation of circulating tET or if ET is actually synthesized by the trout kidney. Furthermore, if ET is synthesized by the kidney, is it for intrarenal regulation or for release into the systemic circulation or both? In regard to systemic distribution of tET, there is an interesting analogy between vasoconstrictor ET and the vasodilator natriuretic peptides. Both are stored and can be potentially released into systemic venous blood, and both have profound, but opposing, effects on gill vascular resistance (this study; Ref. 14). Although it remains to be determined if these two systems are physiologically interactive in branchial vessels, we propose a mechanism whereby natriuretic peptides become significant in mitigating the adverse effects of ET on ventricular afterload. In this model, if renal ET secretion increases to the point where PVA exceeds the mechanical ability of the heart, PVen will increase, which will distend the atrium and stimulate release of natriuretic peptides from the atrium and possibly ventricles (4). Because vasodilation by natriuretic peptides preempts the vasoconstrictory action of most peptide agonists in trout vessels, including ET (Ref. 14; Olson, unpublished observation), untoward increases in Rg can be effectively capped when they reach the point where SV is impaired. This may, in fact, account for the rapid reduction in Rg and restoration of CO after an ET-1 bolus despite continued ventral aortic hypertension (Fig. 1).

Perspectives

The present and previous studies (5, 15, 16, 21) clearly support a role for ET in blood pressure and blood volume regulation in fish. Furthermore, the multiplicity of effectors stimulated by ET suggests that this peptide was incorporated into cardiovascular function early on in vertebrate phylogeny. It is also likely that a variety of additional physiological attributes of this peptide family remain to be discovered.

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